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(54) Title: IDENTIFYING SMALL ORGANIC MOLECULE LIGANDS FOR BINDING (57) Abstract The present invention is directed to novel methods for rapidly and unambiguously identifying small organic molecule ligands for binding to biological target molecules, wherein those methods take advantage of principles of binding avidity. Small organic molecule ligands identified according to the methods of the present invention may find use, for example, as novel therapeutic drug lead compounds, enzyme inhibitors, labeling compounds, diagnostic reagents, affinity reagents for protein purification, and the like. Biological target molecules include, for example, polypeptides, nucleic acids, carbohydrates, nucleoproteins, glycoproteins, glycolipids and lipoproteins.		

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IDENTIFYING SMALL ORGANIC MOLECULE LIGANDS FOR BINDING

FIELD OF THE INVENTION

The present invention is directed to novel avidity-based methods and compositions for quickly and unambiguously identifying small organic molecule ligands that bind to biological target molecules. Small organic molecule ligands identified according to the methods of the present invention find use, for example, as novel therapeutic drug lead compounds, enzyme inhibitors, labeling compounds, diagnostic reagents, affinity reagents for protein purification, and the like.

BACKGROUND OF THE INVENTION

The primary task in the initial phase of generating novel biological effector molecules is to identify and characterize one or more tightly binding ligand(s) for a given biological target molecule. In this regard, many molecular techniques have been developed and are currently being employed for identifying novel ligands that bind to specific sites on biomolecular targets such as proteins, nucleic acids, carbohydrates, nucleoproteins, glycoproteins, glycolipids and lipoproteins. Many of these techniques exploit the inherent advantages of molecular diversity by employing combinatorial libraries of potential binding compounds in an effort to speed up the identification of functional ligands. For example, combinatorial synthesis of both oligomeric and non-oligomeric libraries of diverse compounds combined with high-throughput screening assays has already provided a useful format for the identification of new lead compounds for binding to chosen molecular targets.

While combinatorial approaches for identifying biological effector molecules have

proven useful in certain applications, these approaches also have some significant disadvantages. For example, often there does not exist an appropriate screening assay which allows one to detect binding of a library member to the target molecule of interest when the library member may bind only weakly to the target. Moreover, even when such screening assays are available, in many cases techniques which allow rapid identification of the actual library member(s) which bind most effectively to the target are not available or provide ambiguous results, making the actual identification and characterization of functional ligand molecules difficult, time-consuming or impossible. Furthermore, many approaches currently employed to identify novel ligands are dependent upon only a single specific chemical reaction type, thereby limiting the usefulness of such approaches to only a narrow range of applications. Finally, many of the approaches currently employed are expensive and extremely time-consuming. Thus, there is a significant interest in developing new methods which allow rapid, efficient and unambiguous identification of small organic molecule ligands for selected biomolecular targets.

For the most part, combinatorial libraries that find use in methods for screening against a target biomolecule comprise molecules that are larger than most small organic compounds. Techniques for assaying for binding of such "larger" molecules to a target biomolecule are known in the art and may often be employed to identify the specific library member(s) that bind to the target. However, when libraries of relatively small organic molecules are screened against a biological target molecule, binding of a library member to the target is often difficult to detect because even the "best" small molecules may bind only weakly. Moreover, even if one is able to detect the binding of a library member to the target, actual identification of the bound compound may be impossible unless methods for deconvolution are available. As such, we herein propose novel methods based upon principles of avidity which significantly enhance the ability to screen libraries of relatively small organic molecules for binding to a biological target.

An interaction between two molecules that are capable of binding to one another is often characterized in terms of the strength with which those molecules attach or interact, i.e., the "affinity" that the molecules have for one another. Generally, the

affinity that one molecule has for another is a measurement of the strength of attachment between the molecules assuming that each of the binding molecules may interact only through a single specific site. However, in reality, binding molecules often have multiple sites through which an interaction with another molecule may occur. In such situations, although the affinity at any one binding site may be unchanged, the overall strength of the attachment must take into account binding at all of the available sites on one or both of the binding partners. This overall strength of attachment is known as the "avidity" and will appear as a stronger apparent affinity at any given binding site. Mathematically, the strength of the avidity increases for each occupied site on a binding molecule. The phenomenon of avidity, therefore, might be exploited in ways so as to not only enhance the ability to screen libraries of small organic molecules for binding to a biological target molecule but also to identify organic molecules that actually bind to the target.

It is, therefore, an object of the present invention to provide novel methods and compositions which exploit the avidity phenomenon and allow the quick and unambiguous identification of organic compounds that are capable of binding to a biological target molecule. Such methods are herein described and are quick, easy to perform and relatively inexpensive as compared to other currently employed methods.

SUMMARY OF THE INVENTION

Applicants herein describe an avidity-based molecular approach for rapidly and efficiently identifying small organic molecule ligands that are capable of interacting with and binding to specific sites on biological target molecules, wherein organic compounds identified by the subject methods as being capable of binding to the biological target may find use, for example, as new small molecule drug leads, enzyme inhibitors, labeling compounds, diagnostic reagents, affinity reagents for protein purification, and the like. The herein described approaches allow one to quickly screen a library of small organic compounds to unambiguously identify those that are capable of binding to a site of interest on a biomolecular target. The small organic molecule ligands identified by the methods described herein may themselves be employed for numerous

applications, or may be coupled together in a variety of different combinations using one or more linker elements to provide novel binding molecules.

With specific regard to the above, one embodiment of the present invention is directed to a method for identifying an organic molecule ligand that binds to a site of interest on a biological target molecule, wherein the method comprises the steps of:

- (a) identifying or selecting a biological target molecule that comprises a site of interest to which the organic molecule ligand may potentially bind;
- (b) obtaining a multimeric form of the biological target molecule, wherein the multimeric form comprises at least two linked biological target molecules and a plurality of the sites of interest;
- (c) contacting or combining the multimeric form with at least first and second members of a library of organic compounds that are potentially capable of binding to the site of interest, wherein at least two of the first members of said library bind to the sites of interest of the multimeric form; and
- (d) identifying the first member of the library of organic compounds that bound to the site of interest.

As is evident from the above, because an embodiment of the described method employs a multimeric form of a biological target molecule that comprises a plurality of or at least two sites of interest that are capable of binding to a member of an organic molecule library, the strength with which the multimeric form binds to members of a library of small organic compounds is greatly enhanced due to well known avidity principles, thereby enhancing the ability to detect that binding has occurred and to determine which members of the library are capable of binding to the target molecule. In other words, because of the multiple available binding sites on the multimeric form of the biological target molecule (i.e., the plurality of sites of interest), the overall strength of binding between the multimeric form of the target and an organic compound that binds thereto will be greatly enhanced (i.e., avidity) as compared to "single-site" binding (i.e., affinity).

In certain particular embodiments, the biological target molecule is a polypeptide, a nucleic acid, a carbohydrate, a nucleoprotein, a glycopeptide, a glycolipid, or a

lipoprotein, preferably a polypeptide, which may be, for example, an enzyme, a hormone, a transcription factor, a receptor, a ligand for a receptor, a growth factor, an immunoglobulin, a steroid receptor, a nuclear protein, a signal transduction component, an allosteric enzyme regulator, and the like. The multimeric form of the biological target molecule comprises either covalently or non-covalently linked biological target molecules and may be obtained in a variety of ways including, for example, by chemically linking two or more pre-existing biological target molecules, by directly synthesizing a multimeric form of a biological target molecule or, in the case of a polypeptide, by recombinantly expressing a multimeric form of the polypeptide target molecule.

Other embodiments of the herein described methods which employ libraries of organic compounds which comprise aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, thioesters, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds and/or acid chlorides, preferably aldehydes, ketones, primary amines, secondary amines, alcohols, thioesters, disulfides, carboxylic acids, acetals, anilines, diols, amino alcohols and/or epoxides, most preferably aldehydes, ketones, primary amines, secondary amines and/or disulfides.

With regard to the above described method, at least the (a) multimeric form of the biological target molecule or (b) at least first and second organic compound library members may optionally be covalently attached to a solid matrix material prior to combining the multimeric form with the library members to assess binding therebetween.

Another embodiment of the present invention is directed to a method for identifying an organic molecule ligand that binds to a site of interest on a biological

target molecule, wherein the method comprises:

- (a) identifying or selecting a biological target molecule that comprises a site of interest to which the organic molecule ligand may potentially bind;
- (b) combining the biological target molecule with at least first and second members of a library of multivalent organic compounds that are potentially capable of binding to the site of interest, wherein the site of interest of at least two of the biological target molecules binds to the first member of the library of multivalent organic compounds; and
- (c) identifying the first member of the library of multivalent organic compounds that bound to the biological target molecule.

Similar to the above described embodiment that employs a multimeric form of the biological target molecule, because organic compound libraries may be constructed that contain members which are "multivalent" (i.e., members which have two or more small organic molecules linked together either covalently or non-covalently), the members of the organic compound library may also have a plurality of sites available for bonding to the site of interest on a biological target molecule. Therefore, the strength with which the members of the organic compound library bind to the biological target molecule is greatly enhanced due to well known avidity principles, thereby enhancing both the ability to detect the existence of binding as well as to determine which members of the library are capable of binding to the target molecule. In other words, because of the multiple available binding sites present on the organic compound library members, the overall strength of binding between the biological target and an organic compound that binds thereto will be greatly enhanced (i.e., avidity) as compared to "single-site" binding (i.e., affinity).

Another embodiment of the present invention is directed to methods for identifying an organic molecule ligand that binds to a site of interest on a biological target molecule, wherein the method comprises:

- (a) selecting a biological target molecule that comprises a site of interest to which the organic molecule ligand may potentially bind;
- (b) contacting (i) a multimeric form of the biological target molecule which

comprises at least two linked biological target molecules and at least two sites of interest with (ii) at least first and second members of a library of multivalent organic compounds that are potentially capable of binding to the sites of interest, wherein the multimeric form binds to the first member of the library of multivalent organic compounds; and

(c) identifying the first member of the library of multivalent organic compounds that bound to the multimeric form.

Other embodiments of the present invention are directed to solid matrix materials comprising materials having at least first and second members of a library of organic compounds covalently bound thereto. Such materials may, among other things, find use in the presently described methods.

Additional embodiments of the present invention will become evident to the ordinarily skilled artisan upon review of the present specification.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the binding of biotin-dextran conjugates to anti-biotin monoclonal antibodies.

Figure 2 shows the plots for recognition of either biotin, desthiobiotin, unreacted wells and wells blocked with propionic acid.

Figure 3 shows soluble biotin titrated against a constant concentration of α -Biotin antibody and α -Fc-HRP detection antibody to evaluate the amount of free biotin required to compete with either immobilized biotin or immobilized desthiobiotin.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides rapid and efficient methods for identifying small organic molecule ligands that are capable of binding to selected sites on biological target molecules of interest. The organic molecule ligands themselves identified by the subject methods find use, for example, as lead compounds for the development of novel therapeutic drugs, enzyme inhibitors, labeling compounds, diagnostic reagents, affinity reagents for protein purification, and the like, or two or more of the identified

organic molecule ligands may be coupled together using routine and well known techniques through one or more linker elements to provide novel biomolecule-binding conjugate molecules.

One embodiment of the subject invention is directed to a method for identifying an organic molecule ligand that binds to a site of interest on a biological target molecule. As an initial step in the herein described methods, a biological target molecule is identified or obtained as a target for binding to the small organic molecule compounds screened during the process. Biological target molecules that find use in the present invention include all biological molecules to which a small organic molecule may bind and preferably include, for example, polypeptides, nucleic acids, including both DNA and RNA, carbohydrates, nucleoproteins, glycoproteins, glycolipids, lipoproteins, and the like. The biological target molecules that find use herein may be obtained in a variety of ways, including but not limited to commercially, synthetically, recombinantly, from purification from a natural source of the biological target molecule, and the like.

In a particularly preferred embodiment, the biological target molecule is a polypeptide. Polypeptides that find use herein as targets for binding to organic molecule ligands include virtually any peptide or protein that comprises two or more amino acids and which possesses or is capable of being modified to possess a site of interest that is potentially capable of binding to a small organic molecule. Polypeptides of interest finding use herein may be obtained commercially, chemically, recombinantly, synthetically, by purification from a natural source, or otherwise and, for the most part are proteins, particularly proteins associated with a specific human disease condition, such as cell surface and soluble receptor proteins, such as lymphocyte cell surface receptors, enzymes, such as proteases and thymidylate synthetase, steroid receptors, nuclear proteins, allosteric enzyme inhibitors, clotting factors, serine/threonine kinases and dephosphorylases, threonine kinases and dephosphorylases, bacterial enzymes, fungal enzymes and viral enzymes, signal transduction molecules, transcription factors, proteins associated with DNA and/or RNA synthesis or degradation, immunoglobulins, hormones, receptors for various cytokines including, for example, erythropoietin/EPO,

granulocyte colony stimulating receptor, granulocyte macrophage colony stimulating receptor, thrombopoietin (TPO), IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-11, IL-12, growth hormone, prolactin, human placental lactogen (LPL), CNTF, octostatin, various chemokines and their receptors such as RANTES, MIP1- α , IL-8, various ligands and receptors for tyrosine kinase such as insulin, insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), heregulin- α , and heregulin- β , vascular endothelial growth factor (VEGF), placental growth factor (PLGF), tissue growth factors (TGF- α and TGF- β), other hormones and receptors such as bone morphogenic factors, follicle stimulating hormone (FSH), and luteinizing hormone (LH), tissue necrosis factor (TNF), apoptosis factor-1 and -2 (AP-1 and AP-2), mdm2, proteins and receptors that share 20% or more sequence identity to these, and the like.

A "site of interest" on a biological target molecule may be any site on a target molecule where it is desired that a small organic molecule binds. Sites of interest may be naturally existing on the target or may be artificially introduced into the target using techniques that are routinely employed and well known in the art. In preferred embodiments, the site of interest is an active site of an enzymatic protein or a binding site on a target that binds to another biological target molecule.

In one specific embodiment of the present invention, once a biological target molecule that comprises a site of interest is identified, a multimeric form of that biological target molecule is then obtained. By "multimeric form" is meant that at least two of the biological target molecules of choice are linked, either covalently or non-covalently, preferably covalently, such that the resulting "multimeric" structure comprises multiple copies of the biological target molecule and a plurality of or at least two of the site of interest. The multimeric form of the target molecule may be prepared or obtained in a number of ways including, for example, by chemically crosslinking two or more biological target molecules using any of a number of known chemical crosslinkers, biotin/streptavidin, immunoglobulin-mediated linkage, and the like, by artificially synthesizing a multimeric form of a biological target molecule or, in the case of polypeptide targets, by recombinantly expressing a multimeric form of the target polypeptide. Multimeric forms may also be obtained by covalent bond formation

between two or more chemically reactive groups present on the target molecules. Techniques for obtaining a multimeric form of a biological target molecule are well known in the art and are found in general textbooks such as, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, 1989) and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley-Interscience 1991.

Since a biological target molecule will comprise at least one of the site of interest, the multimeric form of that biological target molecule will possess a plurality of or at least two of the site of interest against which a library of small organic compounds may be screened for binding. Techniques for preparing a multimeric form of a chosen biological target molecule are known in the art and may be employed herein in a routine manner.

A multimeric form of a biological target molecule of interest will comprise at least about 2 linked biological target molecules, often from about 2 to about 200 linked biological target molecules, more often from about 2 to about 100 linked biological target molecules, usually from about 2 to about 50 linked biological target molecules, more usually from about 2 to about 20 linked biological target molecules, preferably from about 2 to about 10 linked biological molecules and more preferably from about 2 to about 6 linked biological molecules. The biological target molecules will be linked either covalently or non-covalently to provide the multimeric form, preferably covalently using well known molecular linking techniques.

In a particular embodiment of the present invention, the multimeric form of the biological target molecule obtained as described above will be combined with or contacted with at least first and second members of a library of organic compounds that are potentially capable of binding to the sites of interest on the multimeric form of the target molecule. Organic compounds will be "potentially capable of binding to a site of interest" if they possess a size or structure which is compatible with the site of interest on the biological target, thereby allowing binding therebetween. The step of combining the multimeric form and the at least first and second organic compound library members will be performed under conditions which are capable of allowing binding to

occur therebetween, wherein those conditions will depend upon the nature of the components of the system and may be determined routinely and empirically.

The first and second organic compounds that find use preferably are of the same chemical class (e.g., are all aldehydes, are all amines, etc.) but may also be of different chemical classes. The library of organic compounds to be screened against the biological target molecule or multimeric form thereof may be obtained in a variety of ways including, for example, through commercial and non-commercial sources, by synthesizing such compounds using standard chemical synthesis technology or combinatorial synthesis technology (see Gallop et al., *J. Med. Chem.* 37:1233-1251 (1994), Gordon et al., *J. Med. Chem.* 37:1385-1401 (1994), Czarnik and Ellman, *Acc. Chem. Res.* 29:112-170 (1996), Thompson and Ellman, *Chem. Rev.* 96:555-600 (1996), and Balkenhohl et al., *Angew. Chem. Int Ed.* 35:2288-2337 (1996)), by obtaining such compounds as degradation products from larger precursor compounds, e.g. known therapeutic drugs, large chemical molecules, and the like.

The monovalent "organic compounds" employed in the methods of the present invention will be, for the most part, small chemical molecules that will generally be less than about 2000 daltons in size, usually less than about 1500 daltons in size, more usually less than about 750 daltons in size, preferably less than about 500 daltons in size, often less than about 250 daltons in size and more often less than about 200 daltons in size, although organic molecules larger than 2000 daltons in size may also find use herein. Organic molecules that find use may be employed in the herein described methods as originally obtained from a commercial or non-commercial source (for example, a large number of small organic chemical compounds are readily obtainable from commercial suppliers such as Aldrich Chemical Co., Milwaukee, WI and Sigma Chemical Co., St. Louis, MO) or may be obtained by chemical synthesis.

Organic molecule compounds that find use in the present invention include, for example, aldehydes, ketones, oximes, such as O-alkyl oximes, preferably O-methyl oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, such as N-methylamines, tertiary amines, such as N,N-dimethylamines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, thioesters, disulfides,

carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds, acid chlorides, and the like. In fact, virtually any small organic molecule that is potentially capable of binding to a site of interest on a biological target molecule may find use in the present invention with the proviso that it is sufficiently soluble and stable in aqueous solutions to be tested for its ability to bind to the biological target molecule.

Libraries of organic compounds which find use herein will generally comprise at least 2 organic compounds, often at least about 25 different organic compounds, more often at least about 100 different organic compounds, usually at least about 300 different organic compounds, more usually at least about 500 different organic compounds, preferably at least about 1000 different organic compounds, more preferably at least about 2500 different organic compounds and most preferably at least about 5000 or more different organic compounds. Populations may be selected or constructed such that each individual molecule of the population may be spatially separated from the other molecules of the population (e.g., each member of the library is a separate microtiter well) or two or more members of the population may be combined if methods for deconvolution are readily available. The methods by which the populations of organic compounds are prepared will not be critical to the invention. Usually, each member of the organic molecule library will be of the same chemical class (i.e., all library members are aldehydes, all library members are primary amines, etc.), however, libraries of organic compounds may also contain molecules from two or more different chemical classes.

Reaction conditions for screening a library of organic compounds against a site of interest-containing biological target molecule will be dependent upon the nature of the site of interest and the chemical nature of the chosen library of organic compounds and can be determined by the skilled artisan in an empirical manner. For the step of screening a population of organic molecules to identify those that bind to a target

polypeptide, it will be well within the skill level in the art to determine the concentration of the organic molecules to be employed in the binding assay.

Because the presently described methods take advantage of the principle of binding avidity, covalent bond formation between chemically reactive groups on the biological target and member(s) of an organic compound library is not desired when those components are combined. Thus, in a particularly preferred embodiment of the present invention, because the members of the organic molecule library and the biological target molecules may both possess chemically reactive groups which potentially would allow undesired covalent bond formation therebetween, the chemically reactive groups on either or both of the biological target molecule or the organic compound library members, preferably the biological target molecule, may be "capped" by treatment with a "capping agent" prior to combining the target and the organic compound library members. By "capping" chemically reactive groups on a target molecule or organic library members, preferably a target molecule, is meant that one or more of the available chemically reactive groups are altered such that they no longer are capable of forming a covalent bond with another chemically reactive group. "Capping agents" that find use for altering chemically reactive groups so as to prevent those groups from participating in covalent bond formation are well known in the art and may be routinely employed herein. Preferably, the biological target and/or the organic compound library members will be chosen such that covalent bond formation therebetween is not possible.

The methods of the present invention are based upon principles of avidity in that at least one of the (a) biological target molecule or multimeric form thereof and/or (b) organic compound library members comprises at least two available sites for binding to its counterpart molecule. By exploiting the phenomenon of avidity, the strength of attachment between the target molecule and the organic compound library members is increased to such an extent so as to enhance or facilitate the ability to not only detect binding between the target and library member(s), but also to allow easy identification of the actual library member that bound to the target biomolecule. In other words, avidity allows for the identification of organic compounds that bind to the target when

they otherwise bind too weakly to be detected by facile detection. As such, one embodiment of the present invention as described above employs a multimeric form of a biological target molecule that comprises at least two sites of interest that are available for binding to an organic compound library member, thereby exploiting the phenomenon of avidity. Yet other embodiments of the present invention, however, may employ a single biological target molecule or multimeric form thereof and a library of "multivalent" organic compounds that are potentially capable of binding to sites of interest on the target, thereby again exploiting the phenomenon of avidity. By "multivalent" when used to describe an organic compound is meant that the compound possesses at least two chemical structures, preferably at least two of the same chemical structures, that are capable of binding to a site of interest on a biological target molecule. This, in one embodiment, at least two organic compounds as described above are linked together, either covalently or non-covalently, so as to produce a "multivalent" molecule that comprises at least two of the organic compound of interest, each of which are available for binding to a site of interest on a target molecule. Preferably, a multivalent organic compound possesses two or more of the same linked organic compound, however, multivalent organic compounds having two or more different linked organic compounds may also find use herein. Multivalent organic compounds for use in the present invention may be obtained in a variety of ways including, for example, commercially, synthetically using well known and routine chemical synthesis techniques, or as degradation products of a larger chemical molecule or other novel techniques.

Specific examples of "multivalent" organic compounds that find use herein may include, for example, dextran whose sugar moieties are oxidized to form aldehyde functionalities, organic compounds linked through a polymeric backbone, dendromers, poly-amino acids such as polylysine which possesses a plurality of amine groups, and the like.

Once a biological target molecule or multimeric form thereof has been combined with members of an organic compound library, multimeric or not, and binding therebetween has been allowed to occur, one must identify the organic compound(s)

that bound to the site(s) of interest on the target molecule. In this regard, many techniques exist for identifying the bound organic compound. For example, the well known technique of mass spectrometry may preferably be employed either alone or in combination with other means for detection for identifying the organic compound ligand that bound to the target of interest. Prior to employing mass spectrometry, one may wish to chemically crosslink the bound organic compound to the target molecule. Techniques employing mass spectrometry are well known in the art and have been employed for a variety of applications (see, e.g., Fitzgerald and Siuzdak, *Chemistry & Biology* 3:707-715 (1996), Chu et al., *J. Am. Chem. Soc.* 118:7827-7835 (1996), Siuzdak, *Proc. Natl. Acad. Sci USA* 91:11290-11297 (1994), Burlingame et al., *Anal. Chem.* 68:599R-651R (1996), Wu et al., *Chemistry & Biology* 4:653-657 (1997) and Loo et al., *Am. Reports Med. Chem.* 31:319-325 (1996)).

In other embodiments, subsequent to the binding of the library member to the target molecule and covalent crosslinking of the bound components, the target molecule/organic compound conjugate may be directly subjected to mass spectrometry or may be fragmented and the fragments then subjected to mass spectrometry for identification of the organic compound that bound to the target molecule. The success of mass spectrometry analysis of the intact target protein/organic compound conjugate or fragments thereof will depend upon the nature of the target molecule and can be determined on an empirical basis.

In addition to the use of mass spectrometry, one may employ a variety of other techniques to identify the organic compound that bound to the biological target molecule of interest. For example, one may employ various chromatographic techniques such as liquid chromatography, thin layer chromatography, and the like, for separation of the components of the reaction mixture so as to enhance the ability to identify the bound organic molecule. Such chromatographic techniques may be employed in combination with mass spectrometry or separate from mass spectrometry. One may optionally couple a labeled probe (fluorescently, radioactively, or otherwise) to the organic compound library members or biological target molecule so as to facilitate its identification using any of the above techniques. Other techniques that may find use

for identifying the organic compound that bound to the target biomolecule include, for example, nuclear magnetic resonance (NMR), capillary electrophoresis, X-ray crystallography, and the like, all of which will be well known by those skilled in the art.

In a particularly preferred embodiment, the identification of the bound organic compound is facilitated by attaching either or both of (a) the biological target molecule or multimeric form thereof or (b) the organic compound library members or multivalent forms thereof to a solid matrix material prior to the step of combining those components. When the biological target molecule or multimeric form thereof is attached to a solid matrix material during the step of combining that solid phase-linked target with members of an organic compound library, the library members which bind to the site of interest on the target necessarily become themselves linked to the solid matrix material which may then be washed to remove any unbound reaction components, thereby facilitating the subsequent identification of the bound members. Along the same vein, when the organic compound library members or multivalent forms thereof are attached to a solid matrix material during the step of combining those members with the biological target molecule or multimeric form thereof, the target molecules that bind to a solid phase-linked library member necessarily become linked to the solid matrix material which may then be washed to remove unbound contaminants, thereby facilitating the identification of the bound members. In another preferred embodiment, different organic compound library members may be covalently attached to spatially distinct regions of the solid matrix material, wherein binding of the target molecule to any specific region of the solid matrix will then necessarily identify the organic compound being bound.

"Solid matrix materials" that find use in the present invention include all of those solid matrix materials that are known in the art and to which biological target molecules and/or organic compounds may be covalently immobilized including, for example, those materials that are routinely employed in the various types of chromatography, affinity purification, or any other techniques that requires that a ligand or potential ligand molecule be covalently immobilized on a solid substrate. The solid matrix materials employed herein may be organic or inorganic in nature and may be, for example, formed from any resin material which will support the attachment of a biological target

molecule or organic compound as described above. For example, synthetic polymer resins such as poly(phenol-formaldehyde), polyacrylic, or polymethacrylic acid or nitrile, amine-epichlorohydrin copolymers, graft polymers of styrene on polyethylene or polypropylene, poly(2-chloromethyl-1,3-butadiene), poly(vinylaromatic) resins such as those derived from styrene, alpha-methylstyrene, chlorostyrene, chloromethylstyrene, vinyltoluene, vinylnaphthalene or vinylpyridine, corresponding esters of methacrylic acid, styrene, vinyltoluene, vinylnaphthalene, and similar unsaturated monomers, monovinylidene monomers including the monovinylidene ring-containing nitrogen heterocyclic compounds and copolymers of the above monomers are all suitable. Techniques for the preparation of such solid matrix materials may be found, for example, in Ikada et al., *Journal of Polymer Science* 12:1829-1839 (1974) or as described in U.S. Patent No. 4,382,124 to Meitzner et al. Other techniques for the synthesis of such solid matrix materials can be found in U.S. Patent Nos. 3,915,642, 3,918,906, 3,920,398, 3,925,019 and the monograph "Dowex: Ion Exchange" 3rd. edition, (1964) published by the Dow Chemical Company, Midland, Michigan.

Additional solid matrix materials that find use in the present invention include, for example, biacore, gold-plated carboxymethylated dextran and other gold films, glass or glass containing matrices, and the like. Preferably, the molecule being immobilized on the solid matrix material and the solid matrix material possess or are modified to possess compatible chemical functionalities such that covalent bonding therebetween may be easily accomplished. In this regard, techniques for immobilizing ligands on a solid matrix material are well known in the art and will depend upon the chemical nature of the components being linked. Detailed conditions for immobilizing ligands onto solid matrix materials may be determined in an empirical manner without undue experimentation. Examples of linkages and chemistries that may be employed for covalently linking a target molecule or organic compound library members to a solid matrix material include, for example, linkage through sulfhydryl groups, linkage through NHS-ester groups, reductive aminations between aldehydes and ketones and amines (March, *Advanced Organic Chemistry*, John Wiley & Sons, New York, 4th edition, 1992, pp.898-900), alternative methods for preparing amines (March et al., *supra*, p. 1276),

reactions between aldehydes and ketones and hydrazine derivatives to give hydrazones and hydrazone derivatives such as semicarbazones (March et al., *supra*, pp.904-906), amide bond formation (March et al., *supra*, p.1275), formation of ureas (March et al., *supra*, p.1299), formation of thiocarbamates (March et al., *supra*, p.892), formation of carbamates (March et al., *supra*, p.1280), formation of sulfonamides (March et al., *supra*, p.1296), formation of thioethers (March et al., *supra*, p.1297), formation of disulfides (March et al., *supra*, p.1284), formation of ethers (March et al., *supra*, p.1285), formation of esters (March et al., *supra*, p.1281), additions to epoxides (March et al., *supra*, p.368), additions to aziridines (March et al., *supra*, p.368), formation of acetals and ketals (March et al., *supra*, p.1269), formation of carbonates (March et al., *supra*, p.392), formation of enamines (March et al., *supra*, p.1284), metathesis of alkenes (March et al., *supra*, pp.1146-1148 and Grubbs et al., *Acc. Chem. Res.* 28:446-452 (1995)), transition metal-catalyzed couplings of aryl halides and sulfonates with alkenes and acetylenes (e.g., Heck reactions) (March et al., *supra*, pp.717-178), the reaction of aryl halides and sulfonates with organometallic reagents (March et al., *supra*, p.662), such as organoboron (Miyaura et al., *Chem. Rev.*, 95:2457 (1995)), organotin, and organozinc reagents, formation of oxazolidines (Ede et al., *Tetrahedron Letts.* 38:7119-7122 (1997)), formation of thiazolidines (Patek et al., *Tetrahedron Letts.* 36:2227-2230 (1995)), amines linked through amidine groups by coupling amines through imidoesters (Davies et al., *Canadian J. Biochem.* 50:416-422 (1972)), and the like. In fact, covalent immobilization of a target molecule or organic molecule library members to a solid matrix material may be accomplished if any compatible chemically reactive groups exist therebetween.

An additional aspect that facilitates the ability to detect binding between the biological target and organic compound library members is to increase the density or local concentration of the solid matrix bound component, preferably the density of the organic molecule library components as bound to the solid matrix. For example, in embodiments where organic compound library members are covalently attached to a solid matrix material and a multimeric form of a biological target molecule is passed over the solid matrix to allow binding therebetween, one may facilitate the ability to

detect binding and to identify the bound components by employing a relatively high local concentration of library members in the matrix. Such concentrations may be empirically determined.

Additional embodiments of the present invention are directed to solid matrix materials having at least first and second members of a library of organic compounds or multivalent organic compounds covalently bound thereto. Such solid matrix materials will find use, for example, in the methods of the present invention.

EXPERIMENT 1

To determine whether activated dextrans would be a suitable soluble scaffold for polyvalent display of small molecules, biotin was conjugated to aldehyde-activated dextrans and tested for avidity in binding to immobilized anti-biotin monoclonal antibodies. To examine the independence of avidity on valency of display, dextrans were prepared having variable numbers of conjugated biotins per molecule, and these were tested in an anti-biotin binding assay.

Preparation of biotin-dextran conjugates

Aldehyde-activated dextran (Pierce Biochemicals) was reacted with mixtures (10 total equivalents per aldehyde group) of varying ratios of 1-aminopropoxylamine (serving as a bifunctional linker) and methoxylamine (serving as a capping agent), in 0.3 M sodium acetate, pH 4.7, for 16 h at room temperature. Reactions were desalted through NAP-5 columns (Pharmacia) into PBS buffer (10 mM sodium phosphate, 150 mM NaCl, pH 7.4), and amine contents determined by Fluoraldehyde assay (Pierce). The dextran solutions were treated with 5 equivalents (per maximal concentration of amine linker + 20 mM) of the sulfo-N-hydroxysuccinamide ester of biotin (which was prepared by reaction of biotin with 1 equivalent each of EDC and sulfo-NHS in DMSO for 1 hour at room temperature) overnight at 4°C. The biotin content of each conjugate was determined by titration with HABA-avidin complex, following the manufacturer's instructions (Pierce).

Anti-biotin MAb binding assay

Nunc Maxisorp 96-well plates were precoated with 5 µg/ml goat anti-mouse Fc polyclonal antibody (Boehringer Mannheim; 100 µl/well in 50 mM sodium carbonate buffer, pH 9.6) overnight at 4°C. Wells were blocked with superbloc in PBS (Pierce) for 1 hour at room temperature, washed (PBS plus 0.05% Tween 20), coated with mouse anti-biotin ascites fluid (Sigma, 3.8 µg/ml in total IgG) in binding buffer (superblock plus 0.05% Tween 20) for 1 hour at room temperature, and washed again. Serial dilutions of biotindextran conjugates were added, followed by biotin-horseradish peroxidase (HRP) conjugate at a concentration predetermined to give yield subsaturating binding. After 2 hours at room temperature, wells were washed and assayed for HRP activity. IC_{50} values were determined from 4-parameter fits of displacement plots using Kaleidagraph (Synergy Software). The amine content of the dextrans prior to reaction with sulfo-NHS-biotin were calculated assuming 100% recovery of dextran from the desalting column, and a dextran molecular weight of 40,000.

Results

Plots of the IC_{50} concentrations, on a per biotin-basis (biotin concentrations determined by direct assay), for anti-biotin binding versus the number of amine linkers per dextran precursor to the conjugates are shown in Figure 1. The optimal avidity effect was seen from the conjugate that was derived from the dextran containing 60 amine linkers per molecule, and amounted to an 80-fold decrease in the apparent biotin IC_{50} relative to that of free biotin. The inhibition seen in the dextran sample having no available amine groups is due to the presence of free biotin that arose from incomplete desalting of the biotin conjugates reactions. The per-biotin IC_{50} of this material (64 nM) corresponds well with the IC_{50} determined for free biotin (61 nM).

EXPERIMENT 2

Purpose: To evaluate whether binding of weak affinity ligands can be detected using a protein possessing multiple binding sites. A commercially available anti-Biotin antibody (Sigma, clone BN-34) binds biotin and also a biotin analog known as

desthiobiotin with affinities of 100 nM and 130 μ M respectively. The small molecule ligands biotin and desthiobiotin will be immobilized on a solid support through a covalent linkage. The affinity for desthiobiotin (130 μ M) is substantially weaker than what is expected to be readily detected using a standard ELISA based assay with a 1:1 protein to ligand binding interaction.

Immobilization and multivalent detection of small molecule ligands:

Method A. To a Covalink 96 well plate (Nunc) was added 100 μ L of either biotin-NHS, desthiobiotin-NHS, or NHS-propionate at a concentration of 10 μ M in phosphate buffered saline pH 7.2 (PBS) containing 1% DMSO. After 1 hour incubation at room temperature, the plate was washed on a plate washer and blocked with 0.05% Tween 20 in Superblock (Pierce) for 2 hours. An appropriate concentration of α -biotin IgG (Sigma) together with α -Fc-HRP conjugate IgG (Boehringer) as previously determined to be subsaturating by titration was added for 1 hour. The plate was washed, and TMB substrate (Pierce) was added and signal allowed to develop following the manufacturers instructions (Pierce).

Method B. Amino PEGA resin (0.25 mmol) (Calbiochem) was swelled in dimethylformamide and treated with a premixed solution of biotin (1 mmol), HBTU (1 mmol) and diisopropylethylamine (1.5 mmol) in DMF. After mixing for 1 hour the resin was drained and washed with DMF and dichloromethane. The same method was used to covalently link desthiobiotin and acetic acid. Resin was aliquoted into separate wells of a 96 well polystyrene filter plate and an appropriate concentration of α -biotin IgG (Sigma) together with α -Fc-HRP conjugate IgG (Boehringer) in PBS with 0.05% Tween 20 was added. After 1 hour the resin was drained, washed with PBS containing 0.05% Tween 20, and TMB substrate was added according to the manufacturers instructions (Pierce).

Method C. Glass microscope slides (VWR) were cleaned using a mixture of sulfuric acid and hydrogen peroxide. The slide were treated with a 5% solution of

aminopropyl triethoxy silane in 95% ethanol for 1 hour. After treatment, the slides were washed with ethanol and annealed at 120°C for 2 hours. The slides were derivatized at specific sites by the addition of a 10 μ M solution of either biotin-NHS, desthiobiotin-NHS, or NHS-acetate in PBS containing 1% DMSO for 1 hour. The slides were then washed with water and methanol. Sections of the glass slide were cut into pieces according to where the different small molecules were coupled, these pieces were distributed into a 96 well polystyrene filter plate and an appropriate concentration of α -biotin IgG (Sigma) together with α -Fc-HRP conjugate IgG (Boehringer) in PBS with 0.05% Tween 20 was added. After 1 hour the resin was drained, washed with PBS containing 0.05% Tween 20, and TMB substrate was added according to the manufacturers instructions (Pierce).

Results

Covalink plates derivatized as described in Method A were first titrated against the α -Biotin antibody. Figure 2 below shows the plots for recognition of either biotin, desthiobiotin, unreacted wells and wells blocked with propionic acid. The antibody readily detects both the immobilized biotin and the immobilized desthiobiotin at sub nM concentrations. The relatively similar sensitivity for detection of both biotin and desthiobiotin is indicative of the avidity effect. Even though the affinity of the antibody for each of these two ligands differs by 1000 fold, they are both readily recognized. The detection system is potentially tetravalent as the α -Fc-HRP detection antibody can dimerize the α -Biotin antibody which already possesses two binding sites. The potential affinity of a tetravalent antibody is well below the sensitivity of this assay which is most likely titrating the affinity of the α -Fc-HRP detection antibody for the α -Biotin antibody. These results indicate multivalent binding systems will give very high observed binding sensitivity and may require specialized assays to accurately quantitate the results.

In Figure 3, soluble biotin is titrated against a constant concentration of α -Biotin antibody and α -Fc-HRP detection antibody to evaluate the amount of free biotin required to compete with either immobilized biotin or immobilized desthiobiotin. As expected, the concentration of soluble biotin (approximately 1 μ M) required to compete

with the immobilized biotin is much higher than the measured affinity of the antibody for biotin (100 nM). The second curve shows the titration of soluble biotin in a well containing immobilized desthiobiotin. Much lower concentrations of soluble biotin (approximately 50 nM) are required to compete with this interaction due to the substantially lower affinity of the antibody for desthiobiotin.

Similar experiments conducted with supports constructed using Method B and Method C gave similar results.

EXPERIMENT 3

Techniques to prepare Multimeric Proteins

Method A. To a solution of IL-4 (50 μ M, MES pH 6.0) was treated with 2 equivalents of biotin-NHS (Pierce) at 4°C for 12 hours. Analysis by mass spectrometry revealed a mixture of approximately equal parts of mono-biotinylated and non-modified IL-4. The protein was separated from unreacted biotin by purification on a NAP-5 column (Pharmacia). The mixture of protein was further purified by incubation with 0.2 equivalents of neutravidin (Pierce) to form the tetravalent complex (4:1 IL-4:neutravidin). The desired tetravalent complex was purified by size exclusion chromatography using a Bio-Silect SEC 125-5 column (Bio-Rad). Additionally, site specific biotinylation was achieved by expression of a cysteine mutant A104C (other sites could easily be used for the same purpose) and reaction with Biotin HPDP (Pierce). This strategy is potentially important to prevent blocking of the target protein binding site through modification.

Method B. Aldehyde-activated dextran (Pierce) was treated with 3 equivalents of pyridyldithiopropionyl hydrazide (PDPH, Pierce), in 50 mM MES pH 6.0 for 12 hours. The reaction was purified using a NAP-5 column (Pharmacia) and the number of thiopyridyl groups incorporated could be quantified by reduction of the disulfide with DTT and monitoring the UV absorbance at 340 nM (thiopyridone). The number of aldehyde sites that were derivatized with the thiopyridyl functionality could be adjusted by adding a capping hydrazide reagent, such as semicarbazide, to react with a certain

percentage of the available aldehydes. The thiopyridyl-dextran was then reacted with IL-4 A104C to generate IL-4 derivatized dextrans. These constructs were purified by size exclusion chromatography using a Bio-Silect SEC 125-5 column (Bio-Rad).

Method C. To prepare a lipid that could be readily derivatized, 10 mg of phosphatidyl ethanolamine (Sigma, P7943) was dissolved in chloroform (2 mL) with 3 equivalents of diisopropylethylamine. To incorporate a thiol specific reactive functionality, 5 mg of a bifunctional maleimide-NHS ester crosslinker (BMPS, Pierce) was added. After 2 hours at room temperature, the reaction was concentrated and purified on a silica gel column. Liposomes were prepared using extrusion techniques with a mixture of the maleimide derivatized lipid at levels of 1% to 5% with phosphatidylcholine making up the remainder. Liposomes were purified by size exclusion chromatography using a Bio-Silect SEC 125-5 column (Bio-Rad). Proteins containing a free cysteine, such as IL-4 A104C or the anti-biotin antibody derivatized with a free thiol using the reagent SATA (Pierce), were covalently linked to the exterior of the liposome through reaction between the thiol and the maleimide.

The foregoing description details specific methods which can be employed to practice the present invention. Having detailed such specific methods, those skilled in the art will well enough know how to devise alternative reliable methods at arriving at the same information in using the fruits of the present invention. Thus, however, detailed the foregoing may appear in text, it should not be construed as limiting the overall scope thereof; rather, the ambit of the present invention is to be determined only by the lawful construction of the appended claims. All documents cited herein are expressly incorporated by reference.

WHAT IS CLAIMED IS:

1. A method for identifying an organic molecule ligand that binds to a site of interest on a biological target molecule, said method comprising:
 - (a) selecting a biological target molecule that comprises a site of interest to which said organic molecule ligand may potentially bind;
 - (b) contacting a multimeric form of said biological target molecule which comprises at least two linked biological target molecules and at least two sites of interest with at least first and second members of a library of organic compounds that are potentially capable of binding to said sites of interest, at least two of said first members of said library binding to said sites of interest of said multimeric form; and
 - (c) identifying said first member of said library of organic compounds.
2. The method according to Claim 1, wherein said biological target molecule is selected from the group consisting of a polypeptide, a nucleic acid and a carbohydrate.
3. The method according to Claim 1 or 2, wherein said biological target molecule is a polypeptide which is an enzyme, a hormone, a transcription factor, a receptor, a ligand for a receptor, a growth factor or an immunoglobulin.
4. The method according to Claim 1, wherein said site of interest comprises an enzyme active site or a ligand binding site.
5. The method according to Claim 1, wherein said multimeric form of said biological target molecule is obtained by chemically linking at least two of said biological target molecules.
6. The method according to Claim 1, wherein said biological target molecule is a polypeptide and said multimeric form is obtained by recombinant expression thereof.

7. The method according to Claim 1, wherein said multimeric form of said biological target molecule comprises from 2 to about 100 linked biological target molecules.

8. The method according to Claim 1, wherein said multimeric form of said biological target molecule comprises from 2 to about 10 linked biological target molecules.

9. The method according to Claim 1, wherein said multimeric form of said biological target molecule comprises at least two covalently linked biological target molecules.

10. The method according to Claim 1, wherein said multimeric form of said biological target molecule comprises at least two non-covalently linked biological target molecules.

11. The method according to Claim 1, wherein said library of organic compounds comprises aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, thioesters, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds or acid chlorides.

12. The method according to Claim 1, wherein prior to said step of contacting chemically reactive groups on said multimeric form capable of forming a covalent bond with said library members are capped by treatment of said multimeric form with a

capping agent.

13. The method according to Claim 1, wherein during said step of contacting at least said multimeric form or said at least first and second members of said organic compound library are covalently attached to a solid matrix material.

14. The method according to Claim 13, wherein said multimeric form is covalently attached to said solid matrix material.

15. The method according to Claim 13, wherein said at least first and second members of said organic compound library are covalently attached to said solid matrix material.

16. The method according to Claim 15, wherein said at least first and second organic compound library members are covalently attached to spatially distinct regions of said solid matrix material and step (c) is accomplished by determining the spatially distinct region to which said multimeric form binds.

17. The method according to Claim 13 further comprising the step of washing unbound components from said solid matrix material, wherein said washing is performed subsequent to step (b) and prior to step (c).

18. A method for identifying an organic molecule ligand that binds to a site of interest on a biological target molecule, said method comprising:

(a) selecting a biological target molecule that comprises a site of interest to which said organic molecule ligand may potentially bind;

(b) contacting said biological target molecule with at least first and second members of a library of multivalent organic compounds that are potentially capable of binding to said site of interest, wherein the site of interest of at least two of said biological target molecules binds to said first member of said library of multivalent

organic compounds; and (c) identifying said first member of said library of multivalent organic compounds.

19. The method according to Claim 18, wherein said biological target molecule is selected from the group consisting of a polypeptide, a nucleic acid and a carbohydrate.

20. The method according to Claim 18, wherein said site of interest comprises an enzyme active site or a ligand binding site.

21. The method according to Claim 18, wherein prior to said step of contacting chemically reactive groups on said biological target molecule capable of forming a covalent bond with said library members are capped by treatment of said target molecule with a capping agent.

22. The method according to Claim 18, wherein during said step of contacting at least said biological target molecule or said at least first and second multivalent organic compound library members are covalently attached to a solid matrix material.

23. The method according to Claim 22, wherein said at least first and second multivalent organic compound library members are covalently attached to said solid matrix material.

24. The method according to Claim 23, wherein said at least first and second multivalent organic compound library members are covalently attached to spatially distinct regions of said solid matrix material and step (c) is accomplished by determining the spatially distinct region to which said biological target molecule binds.

25. The method according to Claim 22 further comprising the step of washing unbound components from said solid matrix material, wherein said washing is

performed subsequent to step (b) and prior to step (c).

26. A method for identifying an organic molecule ligand that binds to a site of interest on a biological target molecule, said method comprising:

(a) selecting a biological target molecule that comprises a site of interest to which said organic molecule ligand may potentially bind;

(b) contacting (i) a multimeric form of said biological target molecule which comprises at least two linked biological target molecules and at least two sites of interest with (ii) at least first and second members of a library of multivalent organic compounds that are potentially capable of binding to said sites of interest; wherein said multimeric form binds to said first member of said library of multivalent organic compounds; and

(c) identifying said first member of said library of multivalent organic compounds.

27. A solid matrix material having at least first and second members of a library of organic compounds covalently bound thereto.

28. The solid matrix material according to Claim 27, wherein said library members comprises aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, thioesters, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds or acid chlorides.

29. The solid matrix material according to Claim 27, wherein said at least first

and second members of a library of organic compounds are multivalent organic compounds.

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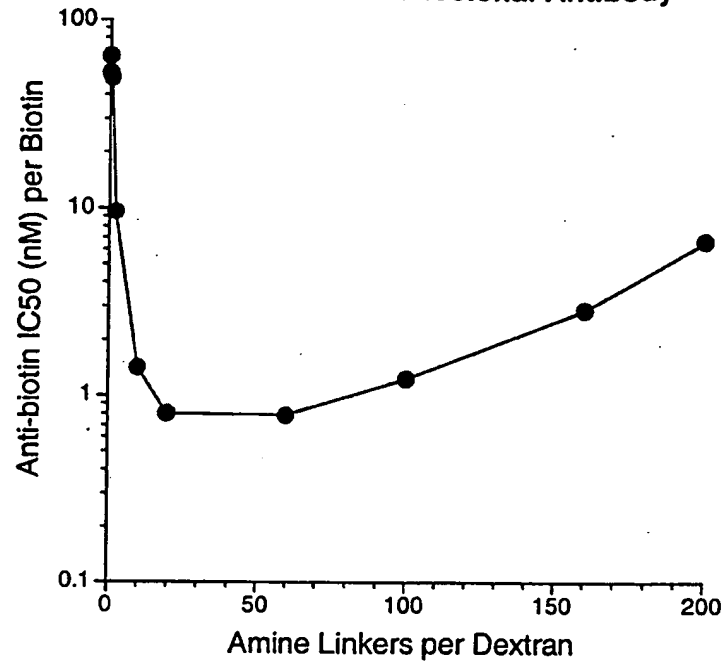
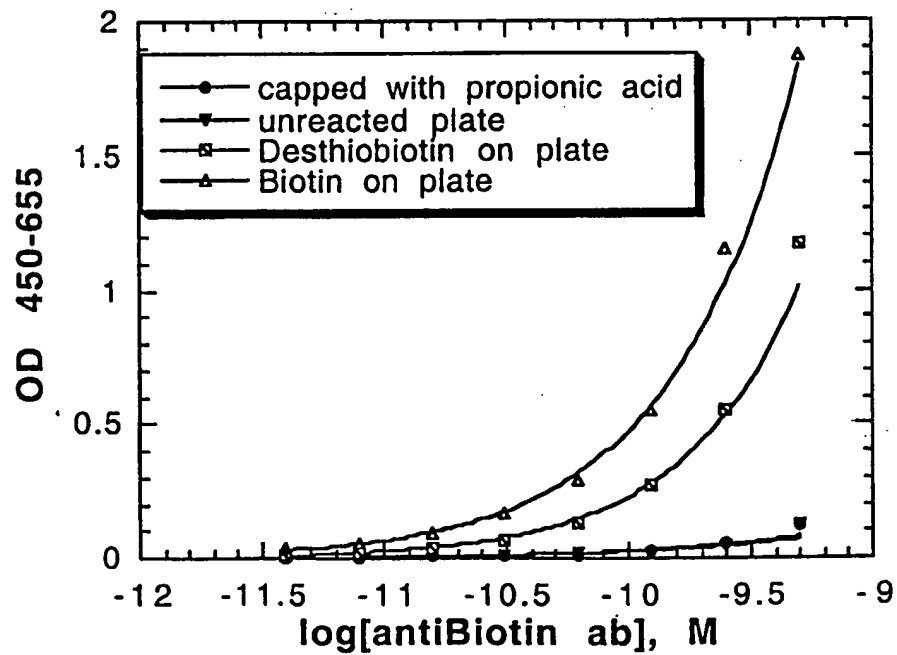
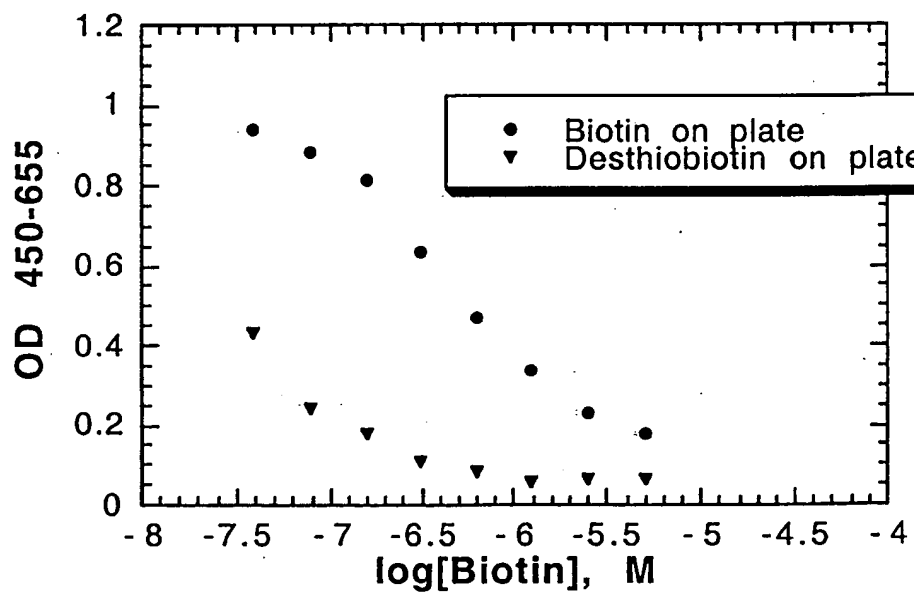
Figure 1. Binding of Biotin-Dextran Conjugates to Anti-Biotin Monoclonal Antibody**Figure 2**

Figure 3

INTERNATIONAL SEARCH REPORT

national Application No

PCT/US 99/30960

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/53 C07B61/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 25146 A (GLAXO GROUP LTD ;DOWER WILLIAM JAMES (US); GATES CHRISTIAN M (US);) 11 June 1998 (1998-06-11) page 47, line 38 -page 48, line 2 ---	1-29
P,X	WO 99 40435 A (NETZER WILLIAM J) 12 August 1999 (1999-08-12) page 55, line 29 - line 33 ---	1-29
X	WO 98 19162 A (FRELINGER JEFFREY A ;NOVALON PHARMACEUTICAL CORP (US); KAY BRIAN K) 7 May 1998 (1998-05-07) page 52, line 31 -page 53, line 6 --- -/--	1-29

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"&" document member of the same patent family

Date of the actual completion of the international search

16 May 2000

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

national Application No

PCT/US 99/30960

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TERRETT N K ET AL: "COMBINATORIAL SYNTHESIS - THE DESIGN OF COMPOUND LIBRARIES AND THEIR APPLICATION TO DRUG DISCOVERY" TETRAHEDRON, NL, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 51, no. 30, 24 July 1995 (1995-07-24), pages 8135-8173, XP000644580 ISSN: 0040-4020 paragraph '0004!	27-29
X	GALLOP M A ET AL: "APPLICATIONS OF COMBINATORIAL TECHNOLOGIES TO DRUG DISCOVERY. 1. BACKGROUND AND PEPTIDE COMBINATORIAL LIBRARIES" JOURNAL OF MEDICINAL CHEMISTRY, US, AMERICAN CHEMICAL SOCIETY. WASHINGTON, vol. 37, no. 9, 29 April 1994 (1994-04-29), pages 1233-1251, XP000195952 ISSN: 0022-2623 figure 3	27-29
X	THOMPSON L A ET AL: "SYNTHESIS AND APPLICATIONS OF SMALL MOLECULE LIBRARIES" CHEMICAL REVIEWS, US, AMERICAN CHEMICAL SOCIETY. EASTON, vol. 96, no. 1, 1 January 1996 (1996-01-01), pages 555-600, XP000652191 ISSN: 0009-2665 page 557, paragraph 6	27-29

INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No

PCT/US 99/30960

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WO 9940435 A	12-08-1999	AU 2590099 A	23-08-1999
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